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This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

Docket No.	14401PRO	Type a plus sign (+) inside this box →	+

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TITLE OF THE INVENTION (280 characters max)

NANOSTRUCTURED THERMOSENSITIVE MEMBRANES AS WOUND DRESSING

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 7 ☒ Drawing(s) Number of Sheets 3

WHICH IS INCORPORATED INTO THE SPECIFICTAION

METHOD OF PAYMENT (check one)

☒ A check or money order is enclosed to cover the Provisional filing fees ☒ The Commissioner is hereby authorized to charge any additional fees and credit any overages to Deposit Account Number: 04-1577	Provisional filing fee amount(s)	$ 160.00

Respectfully submitted,

Signature: Wendy M Slade Date: July 18, 2003

Typed or Printed Name: Wendy M. Slade Registration No.: 53,604

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Invention Description

Nanostructured thermosensitive membranes as wound dressing

This invention relates to nanostructured thermo-sensitive membranes for wound healing. The membranes disclosed herein are transparent, and exhibit thermo-sensitive swelling properties. The lower the temperature, the greater is the swelling ratio. This property would make easy peeling and painless application of the membranes in wound healing. In addition, the transparency of the membranes allows for constant observations of wound while providing a moist wound-healing environment. Moreover, the membranes are thermally stable up to 300 °C, and thus autoclavable and stable during storage and transport. Antibacterial agents and wound healing accelerators can be loaded into the membranes, and sustained release of antibacterial agents and wound healing accelerators can be achieved.

Materials

Methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA) were distilled at reduced pressure. *N*-isopropylacrylamide (NIPAAm) was purified by crystallization (hexane). Ethylene glycol dimethacrylate (EGDMA), and 2,2-dimethoxy-2-phenylacetophenone (DMPA) from Aldrich were used without further purification. ω-methoxy poly(ethylene oxide)$_{40}$ undecyl α-methacrylate macromonomer (C_1-PEO-C_{11}-MA-40) was provided by Prof. Leong-Ming Gan (IMRE). Fluronic68-diacrylate was synthesized in our laboratory.

Methods

Synthesis of fluronic68-diacrylate

The fluoric-68 is dissolved in the dried CH_2Cl_2 with triethylamine. Under nitrogen environment, the methacryloyl chloride was added drop wise into the solution that was magnetically stirred and incubated in an ice bath for half an hour. The mixture was further stirred at room temperature for overnight. The precipitated triethylammonium chloride was filtered, and the excess acryloyl chloride, CH_2Cl_2, and triethylamine were removed by rotary evaporation. The residue was dissolved in distilled chloroform and it was washed twice with saturated sodium bicarbonate solution. The chloroform solution was further washed twice with the saturated brine. A solid product was recovered from the chloroform solution after evaporation. The pure product was obtained by re-precipitating the crude product three times from chloroform against ether.

Fabrication of membranes

The membranes were prepared directly by microemulsion polymerization. Such microemulsions comprise of variable amounts of HEMA, MMA, NIPAAm, ultra-pure water, and of the surfactant C_1-PEO-C_{11}-MA-40 or fluronic68-diacrylate, the cross-linker EGDMA when C_1-PEO-C_{11}-MA-40 is used, as well as the photo-initiator DMPA. Two 20 cm × 20 cm glass plate were washed and dried at room temperature. The glass

surfaces to be contacted with the microemulsions, were then polished using tissue with a small amount of silicon oil. This enabled the easy removal of the polymer membrane after polymerization. About 1 g of each microemulsion was first spread on the glass plate, and then slowly covered with another glass plate to avoid air bubble trapping. Small pieces of thin aluminum foil sheet were used as spacers between the glass plates to regulate the thickness of membranes. The polymerization was carried out in a UV reactor for 6 h. The membranes were immersed in de-ionized water, and the water was changed daily for one week before subjected to further characterization.

Surface Topography

Investigation of the surface topographies of polymeric membranes was conducted on a Thermo Microscope Autoprobe CP Research atomic force microscope (AFM) system (Park Scientific Instrument, Sunnyvale, CA) in contact mode. Conical silicon nitride tips mounted on a silicon cantilever with a force constant of 0.40 N/m were employed. The Si_3N_4 cantilevers (with an integral tip) had a length of 180 μm, width of 38 μm, thickness of 1 μm and resonant frequency of 45 kHz. Each image contains 521 × 512 data points. The surface topographical images were processed using IP2.1 Image Software.

Thermal properties

The decomposition temperatures of polymeric membranes were analyzed by a Perkin Elmer thermogravimetric analyzer (TGA). About 5 – 10 mg sample put in a platinum cell was first kept at 50 °C for one minute, and then it was heated to 800 °C at a rate of 10 °C/min. The temperature range where the sample weight decreased sharply was regarded as the decomposition temperature.

Swelling properties

To measure the equilibrium water content (EWC) and the equilibrium swelling ratios (ESR) of polymeric membranes, pre-weighed dry samples were immersed in distilled water to equilibrium at various temperatures. After the excess surface water was removed with filter paper. The weight of fully swollen samples was recorded. EWC was determined according to the following equation:

$$EWC\ (\%)=(Ws-Wd)/Ws\times 100,$$

where Wd refers to the dry sample weight and Ws is the wet sample weight after swelling equilibrium. EWR was calculated as the ratio of water and dry membrane weight.

Mechanical Properties Measurements

The strain (%) at break, Young's Modulus and tensile strength of polymeric membranes were measured by an Instron microforce tester. Samples with standard size stated in ASTM 638 were used. The tensile rate was 0.25mm/min.

Cell viability studies upon contact with solid membranes

To prepare the membranes for *in vitro* cytotoxicity studies, the membranes were cut into 2×2mm pieces and soaked in PBS solution over night. After being dried in 70°C oven, they are autoclaved for cytotoxicity study. Preliminary study was done on selected membranes. HMN15, HMN16, HMN 17 and HMN19 stand for membranes containing different ratio of monomers. HMN15 is made of monomer ratio of NIPAAm:MMA:HEMA 2:1:1, HMN16 1:1:2, HMN17 1:2:1 and HMN 2.5:1:0.5. Cells were incubated for 24 and 48hours before viable cells were counted. There were 3 replicates for control (cells without specimens) and specimens. Results are expressed as percentage of viable cells relative to control.

Surface topography

As shown in Figure 1, there existed a number of pores on the membrane surface. The size of the pores was less than 100nm.

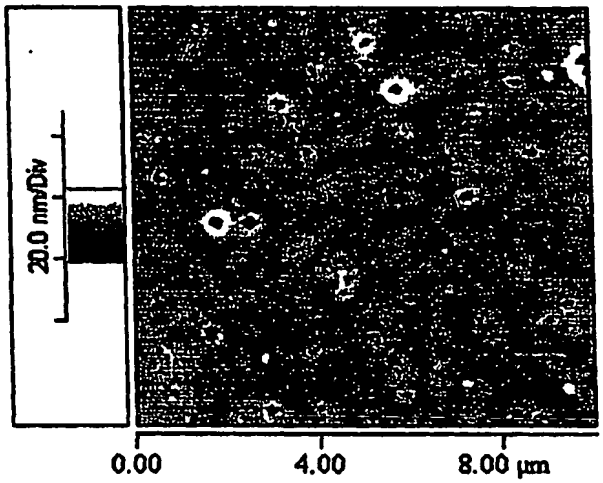

Figure 1 AFM topography image of the HEMA-MMA-NIPAAm membrane

Thermal stability of membranes

All polymeric membranes obtained had a similar decomposition temperature (T_d) ranging from 300 °C to 350 °C. This means that the membranes made by the method described above were thermally stable up to 300°C.

Swelling properties

The equilibrium swelling ratio and water content of membranes, shown in Figure 2 and Table 1 respectively, illustrate temperature dependence. The membranes swelled to higher degree at low temperatures. A discontinuous decrease of swelling ratio took place in the range of 32-37°C, which may be due to LCST of PNIPAAm. When the temperature was above LCST, a phase transition occurred and swelling ratio showed an abrupt decrease. The temperature sensitivity of the membranes was improved with increasing NIPAAm content.

TABLE 1 SWELLING PROPERTIES OF MEMBRANES WITH VARIED COMPOSITIONS

NIPAAm Ratio	MMA ratio	HEMA ratio	EWC (%)			
			4°C	24°C	32°C	37°C
50.0%	25.0%	25.0%	61.4	58.3	53.8	48.0
25.0%	25.0%	50.0%	58.9	52.1	48.3	44.2
25.0%	50.0%	25.0%	55.9	51.0	48.8	43.3
25.0%	62.5%	12.5%	57.3	53.2	50.6	47.1
62.5%	25%	12.5%	57.6	51.2	46.7	40.4
75.0%		25.0%	70.8	63.9	59.1	56.0

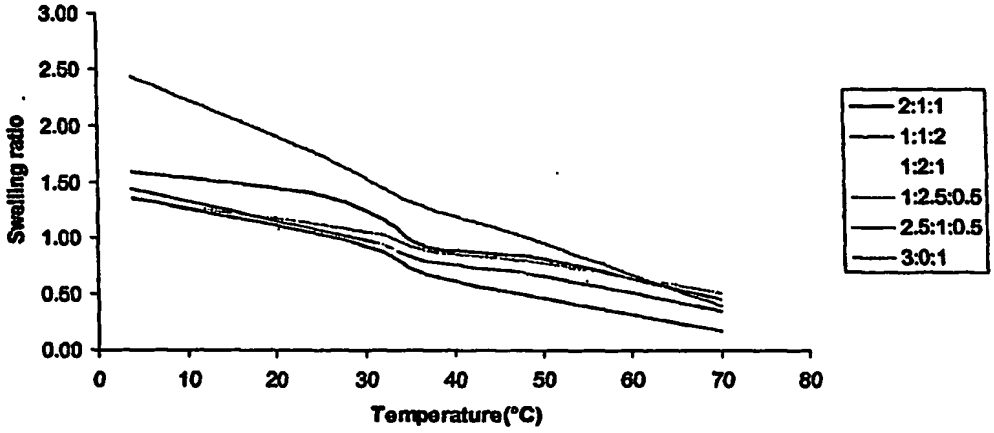

Figure 2 Temperature-responsive swelling properties of membranes

Mechanical properties

TABLE 2 MECHANICAL PROPERTIES OF MEMBRANES DIFFERING IN MONOMER RATIO (N=5)

NIPAAm	MMA	HEMA	Tensile strength (MPa)	Young's modulus (GPa)	% strain at break
75%		25%	10.9±1.2	0.45±0.05	40.2 ±3.81
62.5%	25%	12.5%	4.8±0.78	0.14±0.02	48.6±2.10
50%	25%	25%	5.7±0.51	0.18±0.03	74.4±4.31
25%	25%	50%	6.9±0.55	0.38±0.04	78.0±5.40
25%	50%	25%	5.9±0.21	0.16±0.01	86.9±4.34
25%	62.5%	12.5%	6.2±0.50	0.2±0.03	58.8±7.14

TABLE 3 MECHANICAL PROPERTIES OF SWOLLEN MEMBRANES DIFFERING IN MONOMER RATIO (N=5)

NIPAAm	MMA	HEMA	Tensile strength (MPa)	Young's modulus (GPa)	% strain at break
75%		25%	3.3±0.36	0.19±0.03	17.23±1.87
62.5%	25%	12.5%	2.45±0.25	0.12±0.03	20.1±1.57
50%	25%	25%	3.8±0.43	0.11±0.01	34.2±5.99
25%	25%	50%	4.9±0.55	0.28±0.03	61.0±3.10
25%	50%	25%	5.7±0.21	0.10±0.01	53.9±3.04
25%	62.5%	12.5%	3.9±0.23	0.1±0.02	44.4±3.64

As listed in Table 2, membranes only consisting of NIPAAm and HEMA exhibited the highest tensile strength and Young's modulus but % strain at break was compromised. It may be due to the strong hydrophilic-hydrophilic interactions between polymer chains. With addition of MMA into this system, the membranes lost some of their tensile strength as a whole, but we do see increase in elongation at break. When MMA content was fixed at 25%, with increase of HEMA content, the three mechanical properties were improved significantly. It may suggest that HEMA monomer formed flexible molecular chains upon polymerization in the presence of NIPAAm and MMA, allowing the polymer chains to be stretched to a greater extent. It also demonstrated its ability to resist deformation (Young's modulus). Further increasing MMA content to 62.5% led to less flexible membranes with lower Young's modulus, indicating that polymer chains of MMA is stiffer compared to HEMA or NIPAAm. Therefore, the formulations can be fine-tuned to produce membranes with good mechanical properties suitable for wound dressings.

Mechanical properties of membranes in the swollen state were also investigated in the belief that it would be more favorable if membranes are able to retain their mechanical

properties in the swollen state. It would definitely add to its advantage as a potential wound dressing. As listed in Table 3, most of the materials dramatically lost their mechanical properties after wetted. However, it is noteworthy that two highlighted in red out of those membranes tested retained their tensile strength, and their flexibility remained quite well.

Sustained release of a model drug

Scopolamine and physostigmine were employed as a model drug for study of drug loading and release from the membranes. Scopolamine is a highly water-soluble drug. As shown in Figure 3, scopolamine release was sustained over three days *in vitro*. In particular, drug release from the membranes (NIPAAm/MMA/HEMA=2:1:1 and 1:1:2) was more steady, and initial burst was lower compared to that from the other membranes. It should be noted that the membranes (NIPAAm/MMA/HEMA=1:1:2) possess good mechanical properties even in the swollen state.

In short summary, the membranes are capable to release a drug in a sustained manner, and the composition of the membranes can be fine-tuned to modulate drug release profile.

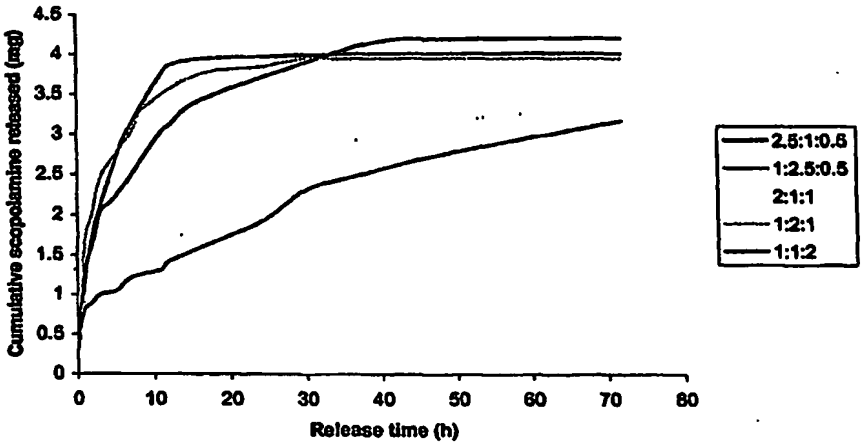

Figure 3 Release profiles of scopolamine-loaded membranes

Study of cytotoxicity

The viability of EL4 (a C57BL/6J mouse lymphoma cell line) after 24 and 48hr incubation with the membranes was tested to evaluate the cytotoxicty of membranes. Cell viability was expressed as a percentage of control. The percentage of control was defined

as the percentage of the number of viable cells after exposure to the membranes relative to the control. The results are shown in Table 4. The membranes with the composition of NIPAAm/MMA/HEMA (50%:25%:25%), (25%:25%:50%) and (25%:50%:25%) provided good viability. The membrane of NIPAAm/MMA/HEMA (62.5%:25%:12.5%) containing the highest NIPAAm content yielded a lower viability, 60.8% after 24 hours incubation. However, it increased to 69.2% after 48 hours contact. This signaled a period of acclimatization of the cells to adapt to the samples and resume normal growth.

TABLE 4 VIABILITY OF EL4 AFTER EXPOSURE TO THE MEMBRANES

Viability	NIPAAm/MMA/ HEMA (50%:25%:25%)	NIPAAm/MMA/ HEMA (25%:25%:50%)	NIPAAm/MMA/ HEMA (25%:50%:25%)	NIPAAm/MMA/HEMA (62.5%:25%:12.5%)
	24 hours incubation			
Percentage of control	105.4%	107.5%	96.1%	60.8%
	48 hours incubation			
Percentage of control	98.4%	104.5%	84.1%	69.2%

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